REMARKS

With entry of this amendment, claims 1-10, 47-50, 61, 63, and 64 are pending in the Application. By this amendment, claims 1, 47, and 61 have been amended and new claims 63 and 64 added for clarity in accordance with the Examiner's suggestions.

Typographical amendments have been presented herein to the specification correcting the amino acid residue change specified by a mutation substituting C for A at nucleotide position 6313. This mutation which encodes a mutation substituting ala for glu at amino acid position 218, which was incorrectly specified as a substitution of asp for glu at position 218 in Tables 37 and 38. Thus, the amendments above correct an obvious error and are consistent with the correct designation of the noted mutation (i.e., substituting ala, not asp, as correctly designated elsewhere in the specification, eg., at Table 39).

All of the amendments presented herein are fully supported by the specification and no new matter has been added to the application. Entry of this amendment is respectfully requested.

Patentability Under 35 U.S.C. § 102 and § 103

Claims 1-7, 9, 10, and 61 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by or in the alternative under 35 U.S.C. § 103(a) as allegedly unpatentable over Murphy et al. (WO 93/21310). In particular, the Examiner asserts that Murphy et al. teach infectious RSV with two or more attenuating mutations, designated as RSV *cpts*-530, RSV *cpts*-248, RSV *cpts*-248/404, RSV *cpts*-248/955, RSV *cpts*-530/1009, and RSV *cpts*-530/1030. In addition, the Examiner relies on Murphy et al. for teaching that RSV *cpts*-248/404 possesses at least three attenuating mutations, and for teaching attenuated RSV formulated

in a vaccine dose of from about $10^3$ to about $10^6$ plaque forming units (PFU).

The Examiner acknowledges that the teachings of Murphy et al. are limited to production of viral mutants by cold adaptation and chemical mutagenesis, whereas the virus (product) of the claimed invention is produced by recombinant processes. However, the Examiner sets forth ground for rejection that Applicants' recitation of "recombinant" RSV in the subject claims does not distinguish the subject matter of the invention from the specific, biologically derived mutant RSV disclosed by Murphy et al. Applicants respectfully traverse the stated grounds of rejection and submit that the disclosure of Murphy et al. neither teaches nor suggests the subject matter of claims 1-7, 9, 10, and 61.

Although Applicants traverse the stated grounds of rejection, the rejection is rendered moot by amendment to the claims hereinabove. In particular, Applicants have amended claim 1 to recite that the claimed, recombinant RSV "has one or more recombinant modifications selected from (i) multiple nucleotide changes in a codon specifying the temperature sensitive substitution, (ii) a restriction marker; (iii) multiple attenuating point mutations adopted from different biologically derived RSV mutant strains, and (iv) a mutation in a 3' promoter element that enhances RSV replication." This language clearly distinguishes the recombinant RSV of the invention from the biologically derived viral mutants (limited to mutants produced by conventional mutagenic and selection methods) of Murphy et al.

Support for the foregoing amendment to claim 1 is provided throughout the specification. In the case of "multiple nucleotide changes in a codon specifying the temperature sensitive substitution", exemplary support is found at, eg., page 124, lines 24-30. There, Applicants teach that:

> [T]he phenotypic stability of these recombinant *cpts*RSV mutants can be enhanced by introducing, where possible, two or more nucleotide substitutions at codons that specify specific amino acids that are known to confer the attenuation phenotype. In this way the stability of the attenuation phenotype can be augmented by site-directed mutagenesis of full-length RSV cDNA.

The Murphy et al. disclosure clearly does not teach or suggest RSV strains which incorporate multiple mutations within a single codon encoding an amino acid change, which novel aspects of the instant invention could not reasonably be practiced according to the conventional mutagenic and selection methods of Murphy et al.

By similar reasoning, Murphy et al. fail to describe or suggest incorporation of a restriction marker in a mutant RSV. Support for this aspect of Applicants' invention is found, for example, at page 10, lines 18-21, page 11, lines 21-26, page 14, lines 11-19, page 15, lines 16-26, page 125, line 34 to page 127, line 12, and page 139, lines 1-16. Thus, Applicants teach that:

> [R]estriction site markers (Fig. 3) were introduced into the antigenome cDNA during the original construction by incorporating the changes into oligonucleotide primers used in RT-PCR. This was done to facilitate assembly, provide a means to identify recombinant virus, and illustrate the ability to introduce changes into infectious RSV.

The disclosure of Murphy et al. fails to provide any suggestion or guidance that would have led to Applicants' recovery of recombinant RSV incorporating restriction markers,

which by definition are not present in biologically derived RSV mutants.

With regard to the reference in claim 1 to "multiple attenuating point mutations adopted from different biologically derived RSV mutant strains", this aspect of the invention clearly falls beyond the scope of disclosure in the Murphy et al. reference. Support for this novel subject matter is provided, eg., at page 15, lines 34-36, page 25, line 26 to page 26, line 2, and at page 124, lines 1-24. Thus, Applicants disclose that:

> [T]he level of attenuation of the *cpts*530/1009 and *cpts*530/1030 mutants can be increased by the specific introduction of one or more of the attenuating mutations in the *cpts*248/404 virus. These examples of combinatorial recombinant viruses, incorporating multiple attenuating mutations from biologically derived mutant strains, overcome many of the difficulties which attend isolation and production of genetically stable, satisfactorily attenuated viruses using conventional approaches. (page 124, lines 26-24).

Similarly, the specification teaches that:

> The present invention provides the ability to distinguish between silent incidental mutations versus those responsible for phenotype differences by introducing the mutations, separately and in various combinations, into the genome or antigenome of infectious RSV clones. This process coupled with evaluation of phenotype characteristics of parental and derivative virus identifies mutations responsible for such desired characteristics as attenuation, temperature

> sensitivity, cold-adaptation, small plaque size, host range restriction, etc. Mutations thus identified are compiled into a "menu" and are then be introduced as desired, singly or in combination, to calibrate a vaccine virus to an appropriate level of attenuation, immunogenicity, genetic resistance to reversion from an attenuated phenotype, etc. (page 25, line 26 to page 26, line 2).

As acknowledged by the Examiner, the disclosure of Murphy et al. is limited to biologically derived mutants of RSV. These mutants are achieved by limited mutagenic and selective methods. Following these limited teachings, persons of ordinary skill in the art would not have a reasonable expectation of success for isolating a RSV mutant incorporating mutations from different biologically derived strains. Reasonable motivation and direction for such achievement is not found in the cited reference, and has instead been provided for the first time by Applicants' disclosure of a successful, recombinant RSV recovery system.

Yet another aspect of the invention set forth in claim 1 relates to novel mutations in a 3' promoter element of recombinant RSV that enhances viral replication. Applicants' specification fully describes and enables such mutations, which dramatically increase viral replication and facilitate recovery of recombinant RSV. One such mutation is described as a substitution of C for G at position four in a 3' promotor element of a cDNA-derived viral antigenome (see specification, eg., at page 14, lines 25-28; page 126, lines 29-30). As in the case of the other novel structural aspects recited in amended claim 1, this and other replication-enhancing mutations in an RSV 3'

promoter element are neither taught nor suggested by the limited disclosure of Murphy et al.

In view of the foregoing, Applicants respectfully urge that the rejection of claims 1-7, 9, 10, and 61 under 35 U.S.C. § 102(b) or, alternatively, § 103(a) should be withdrawn.

Claims 1-3, 10 and 61 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by or in the alternative under 35 U.S.C. § 103(a) as allegedly unpatentable over Crow et al. In particular, Crowe et al. is relied on for teaching the attenuated RSV *cpts* 248 and 248/404 and their formulation into a dose of 6.3 p.f.u. for inoculation of mice. In this context, the teachings of Crowe et al. are no more relevant to the instantly claimed invention than the above-discussed teachings of Murphy et al. In light of the cumulative nature of this reference, and considering the amendment and related discussion set forth above, withdrawal of this rejection is also respectfully requested.

Claims 8 and 47-50 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Murphy et al. in view of Wathen et al. Murphy et al. is relied upon as above for teaching biologically derived RSV mutants with specific mutations inherently incorporated therein. Murphy et al. is further cited for allegedly teaching the desirability of introducing multiple mutations into a single virus for safety in seronegative infants (i.e., to further attenuate the virus and enhance genetic stability). Wathen et al. is relied upon for teaching "recombinantly produced RSV chimeric FG glycoproteins as potential vaccine candidates", and for further teaching processes "for combining nucleic acids using expression vectors to encode for the desired amino acid sequences."

On the basis of the foregoing alleged teachings, the Examiner submits that it would have been *prima facie* obvious to one of ordinary skill in the art to use recombinant techniques, which are allegedly well known in the art and disclosed by Wathen

et al., to make RSV having two desired temperature sensitive mutations fused together into a single genome to improve the safety and stability of vaccine preparations.

Applicants respectfully traverse the foregoing rejections and submit that the combined disclosures of Murphy et al., and Wathen et al. fail to teach or suggest the subject matter of claims 8 and 47-50.

The instantly rejected claims recite recombinant RSV having multiple temperature sensitive mutations (claim 8). Also claimed are compositions comprising expression vectors for expression and recovery of recombinant RSV (claims 47 and 48). Lastly, the rejected claims set forth methods for producing infections RSV from isolated polynucleotide molecules (claims 49 and 50).

The combined disclosures of Murphy et al. and Wathen et al. fail to teach or suggest the inventions of claims 8 and 47-50, which inventions all arise, directly or indirectly, from Applicants' disclosure of novel methods for recovering infectious RSV from cDNA. Neither Murpy et al., nor Wathen et al. disclose such a recovery system, which cannot be compared to simple recombinant methods for making chimeric proteins, as allegedly taught by Wathen et al. On the contrary, as Applicants' disclosure sets forth in detail, recovery systems for producing infectious viruses of any kind from cDNA are complex, and no such systems were previously disclosed for RSV.

Before the development of Applicants' invention, methods for direct genetic manipulation of nonsegmented, negative stranded RNA viruses had only recently begun to be developed. Successful rescue was reported for infectious rabies virus, vesicular stomatitis virus (VSV), measles virus, and Sendai virus from cDNA-encoded antigenomic RNA in the presence of the nucleocapsid N, phosphoprotein P, and large polymerase subunit L (see discussion and cited references at pages 4 and 5 of

specification). However, successful rescue of RSV was complicated by a number of factors.

Specifically, RSV possesses several properties which distinguish it and other members of the genus *Pneumovirus* from better characterized paramyxoviruses of the genera *Paramyxovirus*, *Rubulavirus* and *Morbillivirus*. These differences include a greater number of mRNAs, an unusual gene order at the 3' end of the genome, species-to-species variability in the order of the glycoprotein and M2 genes, a greater diversity in intergenic regions, an attachment protein that exhibits mucin-like characteristics, extensive strain-to-strain sequence diversity, and several proteins not found in other nonsegmented negative stranded RNA viruses.

In view of these and other differences and complicating factors, the Examiner has not provided a *prima facie* showing of obviousness to support the rejection of claims 8 and 47-50. The cited references do not provide a reasonable expectation of success for achieving a successful RSV recovery system. Nor is such a system as described by Applicants disclosed elsewhere in the art pertaining to the invention. In this regard, an important aspect of the instant invention was Applicants' discovery that coexpression of a specific gene M2(ORF1) not previously characterized in any viral recovery system, was required for efficient recovery of RSV. As disclosed in the specification (page 131, line 16 to page 132, line 3.

> RSV was not recovered if any of the plasmids were omitted, as shown in Table 38. The requirement for M2(ORF1) also could be satisfied with the complete gene, M2(ORF1+2), provided the level of its input cDNA was low (0.016 μg per 1.5 X $10^6$ cells (Table 38). At higher levels, the production of virus was greatly reduced, suggesting that an inhibition of minigenome RNA

> synthesis associated with M2(ORF2) also operates on the complete genome during productive infection.
>
> These results showed that the production of infectious RSV was highly dependent on expression of the M2(ORF1) protein in addition to N, P and L. Furthermore, it showed that the optimal method of expression of M2(ORF1) was from an engineered cDNA in which ORF2 had been deleted, although the complete cDNA containing both ORFs also supported the production of RSV.
>
> Thus, the present invention demonstrates that transcription by RSV differs from that of from previously-described nonsegmented negative strand RNA viruses in requiring a fourth protein designated here as M2(ORF1), and previously called 22K or M2 (Collins et al., J. Virol. 54:65-71 (1985)). The M2(ORF1) protein was found to be an RNA polymerase elongation factor essential for processive, sequential transcription. Collins et al., Proc. Natl. Acad. Sci. USA 93:81-85 (1996). This requirement provides the capability, as part of this invention, for introducing specific, predetermined changes into infectious RSV.

These and other teachings of Applicants' disclosure render their recovery system for RSV novel and nonobvious over the prior art. In particular, the cited references by Murphy et al. and Wathen et al. neither teach nor suggest a successful recovery system for RSV. The limited disclosure by Wathen et al. of recombinantly produced chimeric proteins does not allow production of infectious virus, nor of the related compositions

and methods for recombinant RSV production set forth in the instant claims. Accordingly, the rejection of claims 8 and 47-50 is respectfully submitted to be in error.

In view of the above amendments to the claims and accompanying remarks, Applicants believe that each rejection has been addressed and overcome and that the application is now in condition for allowance. Notice to that effect is requested. If for any reason, however, the Examiner feels that a telephone conference would expedite prosecution of the subject application, the Examiner is invited to telephone the undersigned at 206/467-9600.

Respectfully submitted,

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